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ISOETES MICROVELA (ISOETACEAE), A NEW QUILLWORT FROM THE COASTAL PLAIN OF THE SOUTHEASTERN UNITED STATES

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ABSTRACT. *Isoetes microvela*, sp. nov., is described from cytologically confirmed hexaploid populations from the coastal plain of North Carolina. At least one population grows over thinly-buried calcareous bedrock, a rare condition on the predominantly acidic southeastern coastal plain. It is a large quillwort of periodically inundated and scoured stream banks and shallow water. It is characterized by a densely short-crested to reticulate-tuberculate megaspore ornamentation pattern intermediate in appearance between that of *I. appalachiana* and *I. hyemalis*, an exceptionally small velum covering ± 10% of the heavily brown-streaked sporangium, and by obscurely tuberculate microspores. A key to the *Isoetes* of the southeastern coastal plain is presented. *Isoetes microvela* is suspected to represent an allopolyploid derived from the doubling of a sterile triploid hybrid, most likely *I.* ×*bruntonii* (= *I. engelmannii* × *I. hyemalis*) or *I. appalachiana* × *I. engelmannii*. *Isoetes microvela* is a rare species, presently known from only two populations.

Key Words: *Isoetes microvela*, Isoetaceae, pteridophyte, coastal plain endemic, North Carolina

Twenty-four species of *Isoetes* (Isoetaceae) were identified in the *Flora of North America*, 13 of which are known from the southeastern United States (Taylor et al. 1993). A number of additional species have been recognized or described in the relatively short period since then, including *I. virginica* Pfeiffer (*s.str.*), *I. hyemalis* D. F. Brunton, and *I. appalachiana* D. F. Brunton & D. M. Britton. Sterile hybrids between a number of these taxa have recently been described (Montgomery and Taylor 1994; Musselman, Bray, and Knepper 1996, 1997; Musselman et al. 1995), confirming specific distinctions first recognized by other morphological and cytological evidence.

These taxa and the apparent existence of other undescribed species in this area (Hickey 1997) are, in part, reflections of the elevated level of interest in this genus amongst pteridologists.

They also are testaments to the taxonomic value of systematic cytological investigations of *Isoetes* populations across North America, systematic examination of *Isoetes* megaspores and microspores through Scanning Electron Microscopy (SEM), and the application of molecular and genetic investigative techniques.

Of the 16 quillwort species now known in the southeastern United States, eight are diploids ($2n = 22$), five are tetraploids ($2n = 44$), two are hexaploids ($2n = 66$), and one is decaploid ($2n = 110$). Diploid and tetraploid populations are reported for one of these, *Isoetes piedmontana* (Pfeiffer) Reed. In this paper we report the discovery of a third hexaploid taxon and offer evidence that it represents a previously undescribed endemic species of the Atlantic Coastal Plain.

MATERIALS AND METHODS

Extensive field investigations and collecting throughout the southeastern United States by Brunton since 1990 as part of ongoing systematic studies of *Isoetes* in North America have included efforts to re-locate populations of unknown or ambiguous taxa. Cytological investigations of a selection of southeastern *Isoetes* populations have been conducted by Britton during this period. Over 1500 herbarium specimens of *Isoetes* from the southeastern United States in CAN, DFB (D. F. Brunton personal herbarium), DUKE, FLAS, FSU, GA, MICH, NCSC, NCU, NYS, OAC, PH, PSU, UNA, UNCC, USF, VDB, and VPI as well as selected specimens from GH, MO, NY, and US, have been studied. Scanning electron micrographs were taken of selected samples using the standard methods of Britton and Brunton (1989, 1992).

Microspores were measured in Euparol, as described by Britton (1991). Megaspore widths (to the outer edges of spore ornamentation) were measured at a magnification of 40× or 50× on SEM stubs or in sporewells (Brunton 1990) using a binocular stereo microscope equipped with an ocular micrometer.

Chromosome counts were obtained from a selection of coastal plain *Isoetes* populations. Plants from each population were grown in distilled water in a growth cabinet. The developing root tips were excised and pretreated in aqueous paradichlorobenzene (PDB) at room temperature for four hours. They then were washed in distilled water, fixed in acetic alcohol (3:1 absolute ethyl alcohol to glacial acetic acid) for 30 minutes or more, hy-

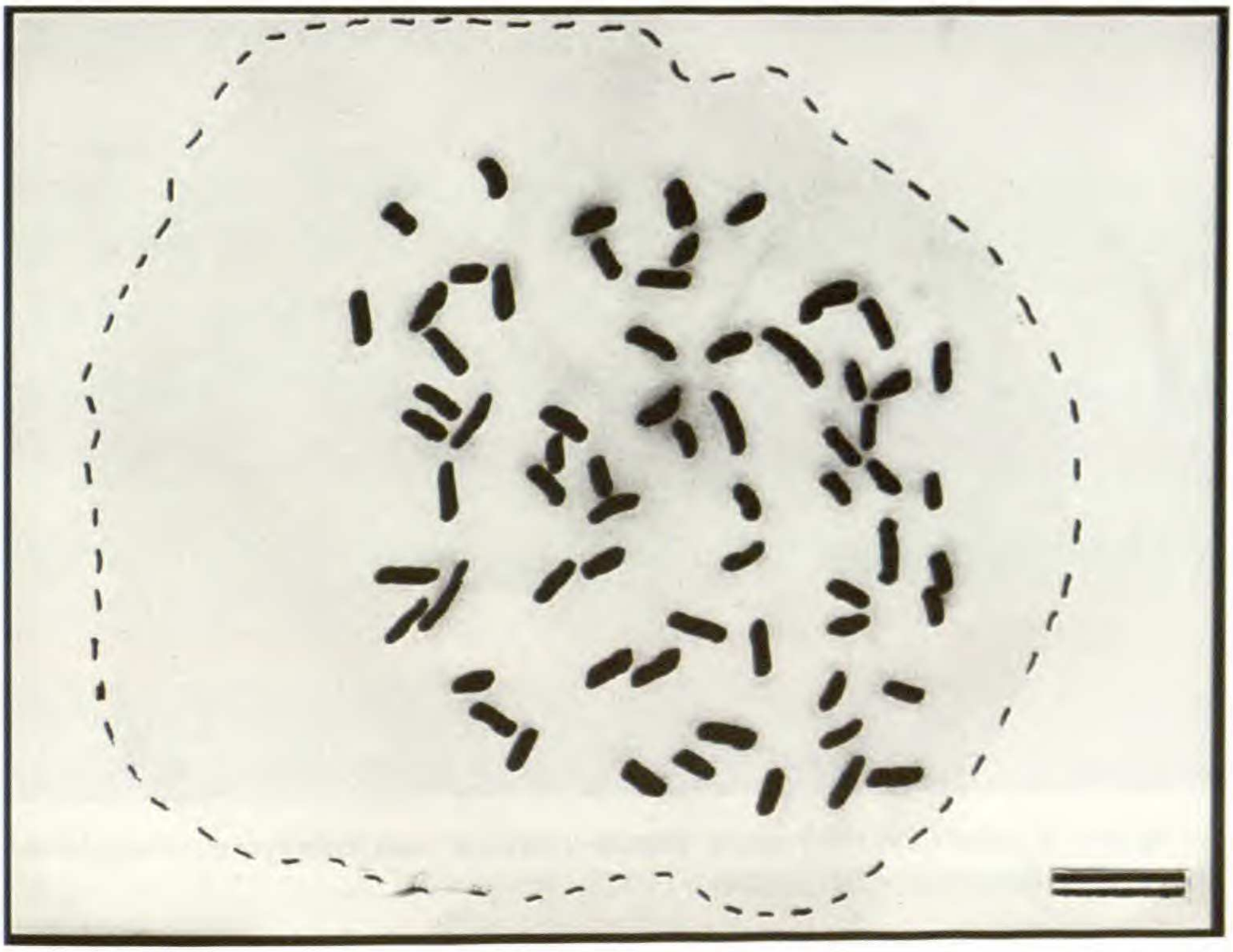

Figure 1. Interpretive drawing of photomicrograph of mitotic root tip plate of *Isoetes microvela*, $2n = 66$ (*D. F. Brunton and K. L. McIntosh 12,599* [OAC]). Scale bar = 5 μm.

drolyzed in Warmke's solution (1:1 concentrated HCl to absolute ethyl alcohol) for 7–10 minutes at room temperature, and stained in leucobasic fuchsin (Feulgen) for two hours. The meristems were squashed under a cover glass in 45% acetocarmine stain and examined.

RESULTS

An examination of herbarium specimens detected populations of large-spored *Isoetes engelmannii* A. Br. (*s.lat.*) quillworts along the coastal plain of North Carolina which could not be attributed to any existing taxon. Cytological determination of material from two North Carolina populations (Maysville, Onslow County, and Bolivia, Brunswick County; *D. F. Brunton and K. L. McIntosh 12,179* and *13,227*, respectively) indicate that they are hexaploid ($2n = 66$; Figure 1).

The two described southeastern hexaploid species, *Isoetes georgiana* Luebke and *I. boomii* Luebke, are closely related in-

Figure 2. *Isoetes microvela* plants (arrows) on emergent river bank, Maysville, Jones Co., NC (2 July 1996).

land endemics of southern and south-central Georgia (Brunton and Britton 1996a; Luebke 1992). The coastal hexaploid populations were found to exhibit consistent and distinctive morphological characteristics which readily separate them from the inland hexaploid populations. The following describes the distinctive morphological characteristics of this previously unknown coastal hexaploid.

Gross morphology. The coastal hexaploid is a large quillwort, with flaccid or strongly reflexed dark, dull olive-green leaves extending (when mature) 35 cm or more from the two-lobed corm (Figure 2). In cross-section, the leaves are ovate and are almost completely occupied by four large, thin-walled, quadrangular air chambers, the abaxial chambers being slightly larger than the adaxial. The air chambers in the leaves of the inland hexaploid species appear to be smaller, more circular, and have thicker walls. Two plants in the Maysville population were found to be joined by a short rhizome-like connection between their corms. Such a "sister" plant arrangement has previously been reported with *Isoetes hyemalis* in North Carolina (Brunton et al. 1994) and in Virginia (R. Bray, pers. comm.). In gross appearance the coastal hexaploid is not unlike many plants of the tetraploids

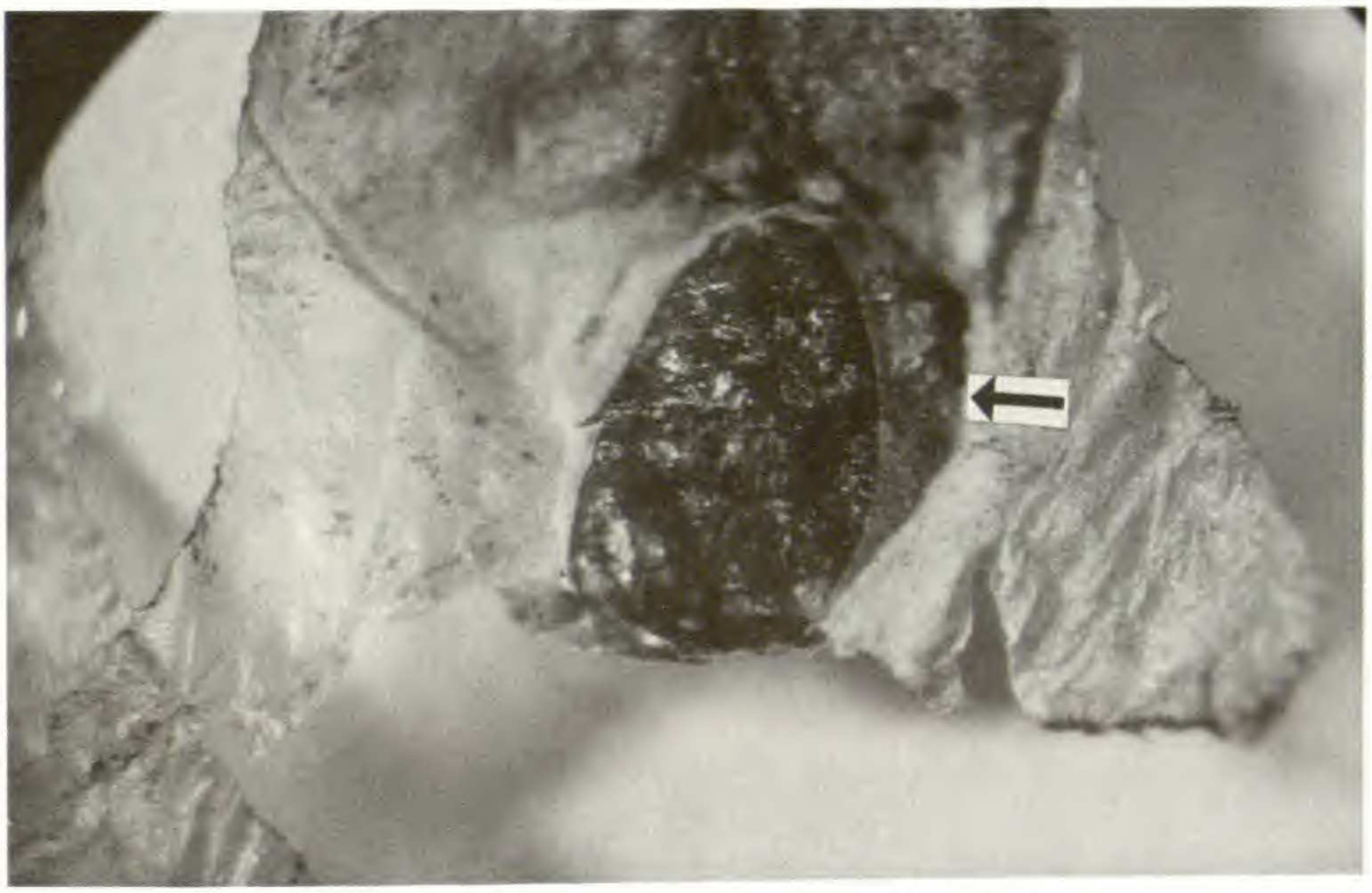

Figure 3. Sporangium of *Isoetes microvela* (*D. F. Brunton and K. L. McIntosh 13,227*); note dark sporangium (streaked) and short velum coverage (arrow).

I. hyemalis and *I. appalachiana* which we have examined from populations along the Carolina and Virginia coastal plain.

The leaf bases of this taxon are pale. Sporangia are topped by a 3.0–4.5 mm long, triangular-lanceolate ligule. The sporangium surface is heavily brown-streaked. The opaque velum is exceptionally narrow, extending across about 10% of the sporangium (Figure 3). This produces the largest fenestra (exposed "window" over the sporangium) of any of the southeastern polyploid species. It is substantially larger than the fenestra created by the 22–60% velum coverage of the Georgia hexaploids or even the 15–25% velum coverage usually observed with the tetraploids *Isoetes hyemalis* or *I. appalachiana* (Brunton and Britton 1996a, 1997; Brunton et al. 1994). Although Russell and Bray (1997) report seasonal variation in the extent of velum coverage of other southeastern hexaploids, fenestra size was consistent on the spring to summer (March to July) coastal hexaploid specimens we examined.

Megaspore size and morphology. Megaspores average ± 530 μm, although individual megaspores up to 700 μm were noted. This average is considerably smaller than that of the Geor-

gia hexaploids (625 μm) and larger than those of the diploid *Isoetes engelmannii* (460 μm). The megaspores of the coastal hexaploid are in the size range of southeastern tetraploid species, *viz.*, *I. appalachiana* (±534 μm) and *I. hyemalis* (±522 μm; Brunton and Britton 1997; Brunton et al. 1994). The similarity of the coastal hexaploid's megaspore size to that of tetraploid taxa rather than other hexaploids is atypical of North American taxa, which normally reflect ploidy level in their spore size (Taylor et al. 1993).

The proximal (triradial) hemisphere of the megaspore is covered by a relatively dense pattern of ragged, usually thin-walled muri (walls), forming crests which join together irregularly (Figure 4e). A very narrow band of short, thin spines borders each of the proximal ridges. This proximal ornamentation pattern is substantially more congested than that of *Isoetes boomii* or *I. georgiana* (Figures 4a, 4i). Neither of the Georgia hexaploids exhibits the fine line of spines seen along the proximal ridges of the coastal hexaploid. This is, however, seen on many *I. appalachiana* megaspores.

The lateral view of the megaspore typically shows a dense, narrow band of thin spines on the distal side of the equatorial ridge (Figure 4f). This characteristic is shared with the tetraploids *Isoetes appalachiana* and *I. hyemalis*. When present, this band is composed of fewer, coarser spines in the inland Georgia hexaploids (Figures 4b, 4j; Brunton and Britton 1996a).

The distal hemisphere is dominated by a raggedly reticulate ornamentation pattern of straight-walled, evenly-topped muri (Figure 4g). As with the proximal hemisphere, this pattern is more congested than that of *Isoetes boomii* or *I. georgiana* (Figures 4c, 4k). It is similar to, but less regularly reticulate than, the distal megaspore ornamentation of *I. appalachiana*. The megaspore ornamentation of the coastal hexaploid usually includes isolated and/or loosely connected tubercles reminiscent of the megaspores of *I. hyemalis*. The overall ornamentation pattern, then, appears intermediate between that of *I. appalachiana* and *I. hyemalis* and is considerably more congested than that of the other hexaploids of the southeastern United States.

Microspore size and morphology. Microspores of the coastal hexaploid are ±29 μm long. This is substantially smaller than the Georgia hexaploids (±37 μm) and approximately equal to

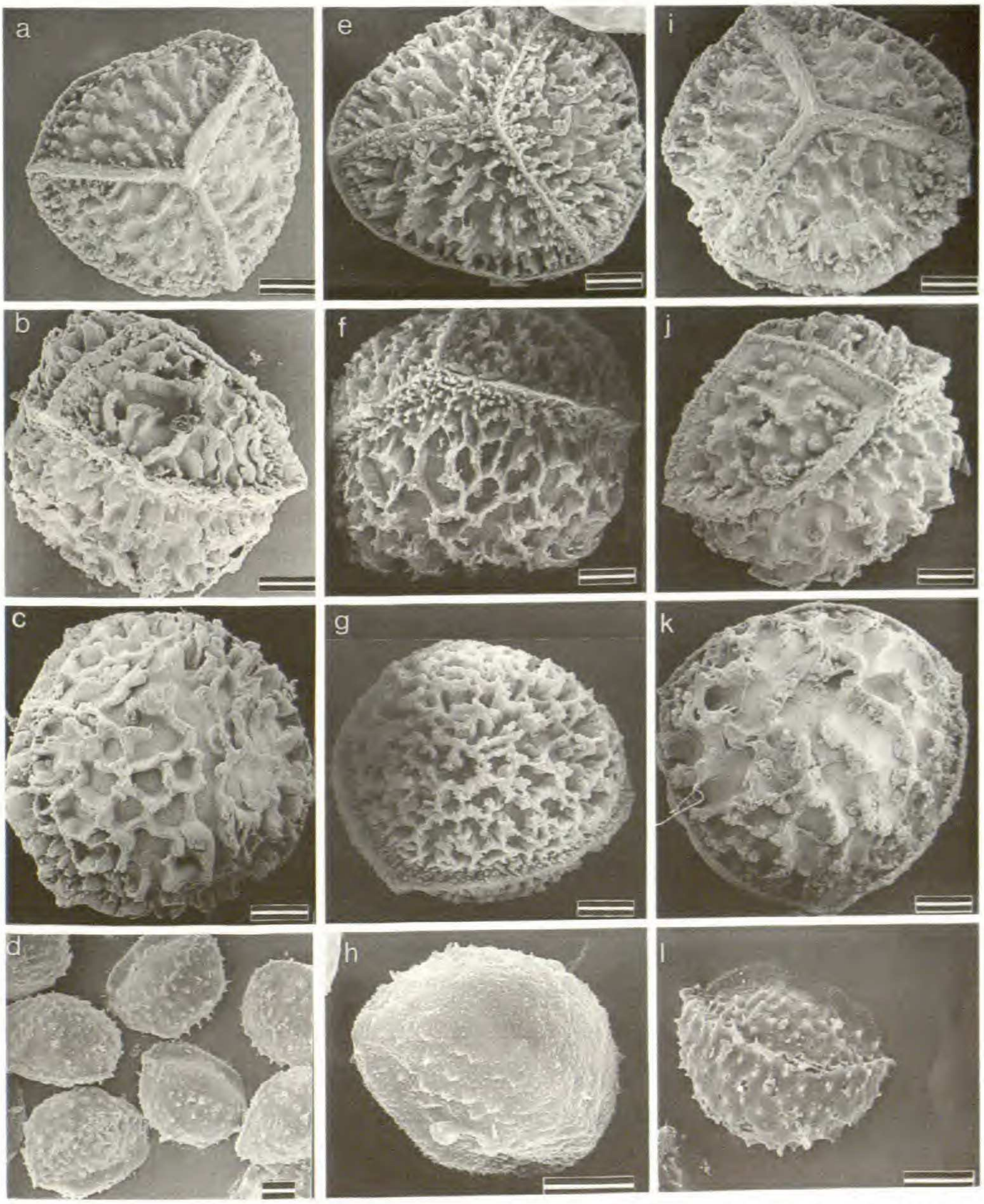

Figure 4. Spores of southeastern hexaploids *Isoetes boomii*, *I. microvela*, and *I. georgiana* (megaspore scale-bar = 100 μm; microspore scale-bar = 10 μm). a–d: *I. boomii* (*D. F. Brunton and K. L. McIntosh 12,063* [OAC]); a: proximal view of megaspore; b: lateral view of megaspore; c: distal view of megaspore; d: group of microspores; e–h: *I. microvela* (megaspores, *D. F. Brunton and K. L. McIntosh 12,213* [OAC]; microspore, *A. E. Radford 5,187* [NCU]); e: proximal view of megaspore; f: lateral view of megaspore; g: distal view of megaspore; h: microspore; i–l: *I. georgiana* (*D. F. Brunton and K. L. McIntosh 11,550* [OAC]); i: proximal view of megaspore; j: lateral view of megaspore; k: distal view of megaspore; l: microspore.

that of tetraploids such as *Isoetes appalachiana* (±30 μm; Brunton and Britton 1996a, 1997). Surface ornamentation is lacking or consists of small, low, sparsely distributed tubercles (Figure 4h). The Georgia hexaploids, in contrast, are aculeate (covered in short, broad-based prickles; Figures 4d, 4l). The microspores of both *I. appalachiana* and *I. hyemalis* are low tuberculate to echinate (Brunton and Britton 1997; Brunton et al. 1994).

Microspores were rarely observed on plants from either cytologically confirmed population; only one plant with mature microspores has been found in collections from each of the Maysville and Bolivia populations. We have found microsporophylls to be substantially more common on all other southeastern species. Finding virtually entirely microsporangiate populations, in fact, can be an impediment to the identification of some *Isoetes* populations in the southeast.

Site ecology. The coastal hexaploid occupies habitats strongly affected by storm-induced flooding. Both cytologically confirmed populations were found along permanent water courses under the deep shade of deciduous swamp forests. The plants grow in sandy alluvium, usually with little or no associated vascular vegetation in shallow water or (more commonly) on seasonally exposed stream or river banks. At the Maysville site at least, the thinly-buried bedrock is calcareous (sedimentary shellstone); this is a rare occurrence in a region of predominantly heavily weathered, acidic soils (LeBlond et al. 1994; Steila 1993).

The Maysville site was severely scoured by post-hurricane flood waters and waterborne debris in late 1996 and early 1997. This erosion resulted in the loss of a substantial proportion of the thin soil covering from the shellstone bedrock and caused the apparent destruction of many *Isoetes* plants (D. F. Brunton, pers. obs.).

Distribution and status. Two cytologically confirmed populations are found along the North Carolina coast. A small population occurs at Bolivia, Brunswick County. A larger population near Maysville extends along either side of the Jones/Onslow County border (Figure 5). In addition, Musselman, Bray, Heafner, and Knepper (1997) report a hexaploid from Florida which may represent this taxon.

Considering the few known sites and the low number of can-

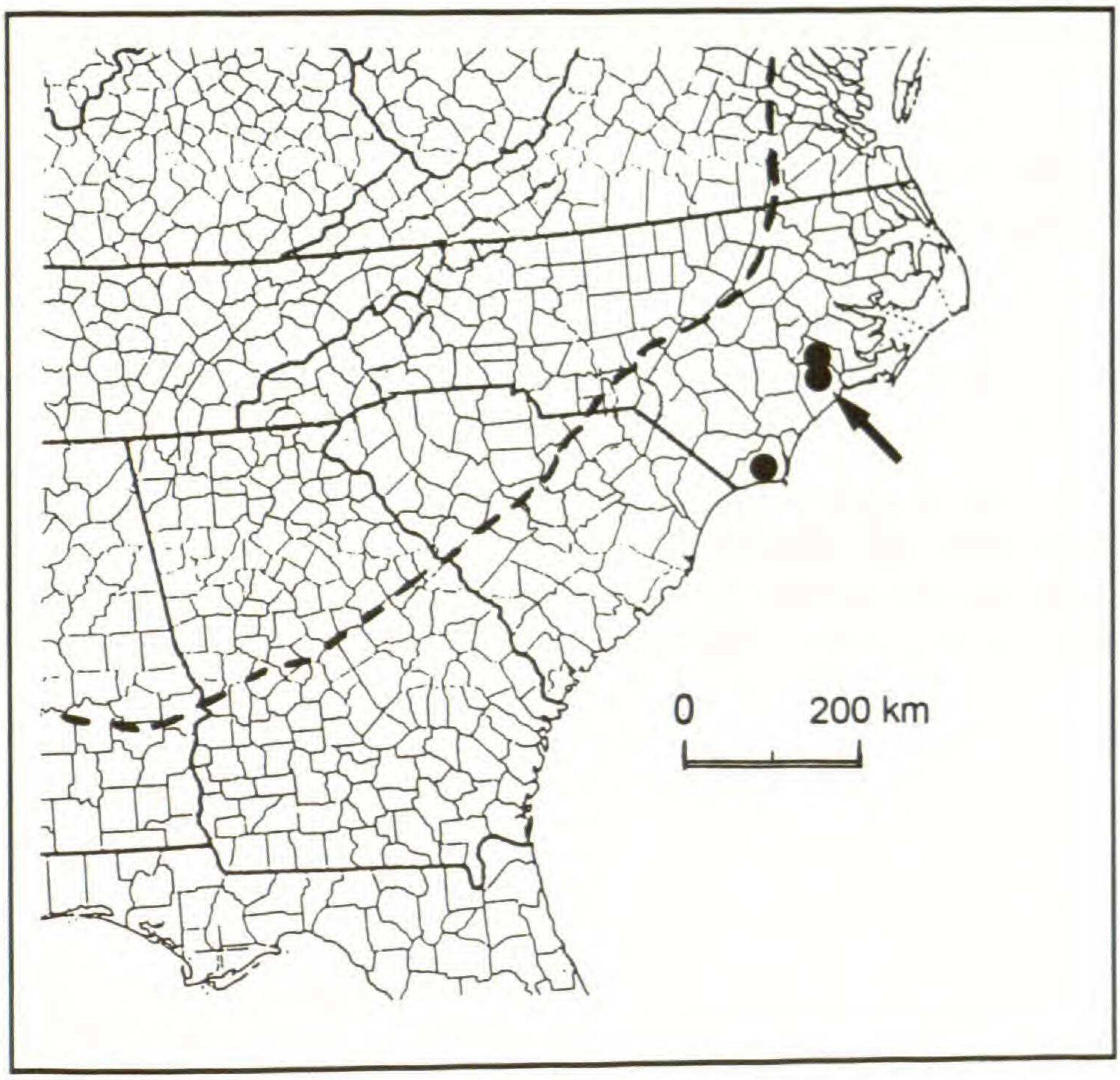

Figure 5. Distribution of *Isoetes microvela* in the southeastern United States (dotted line = boundary between coastal plain and Piedmont; arrow = Type location).

didate populations, this hexaploid appears to represent a legitimately rare Atlantic Coastal Plain endemic of the southeastern United States. A number of similarly rare endemic vascular plants are also known from this area, including the recently described *Carex lutea* LeBlond and *Thalictrum cooleyi* Ahles. The latter two species are found in sandy soil over calcareous bedrock, a rare substrate condition in the southeastern coastal plain (LeBlond et al. 1994) that is also shared by the Maysville population.

DISCUSSION

The characteristics of the coastal plain hexaploid populations indicate they constitute a distinct, small-spored hexaploid species. This new taxon has a megaspore ornamentation intermediate be-

tween that of the tetraploids *Isoetes hyemalis* and *I. appalachiana*, an exceptionally short velum, and plain to obscurely ornamented (and rarely observed) microspores. Accordingly, the following binomial is proposed.

Isoetes microvela D. F. Brunton, *sp. nov.* (Figures 1, 2, 3, and 4e–h). TYPE: U.S.A. North Carolina: Onslow Co., Maysville, banks of White Oak River, 21 May 1995, *D. F. Brunton and K. L. McIntosh 12,179* (HOLOTYPE: OAC; ISOTYPES: MIL, US, MICH, MU, DAO, DFB, ODU).

Herba amphibia e cormo bilobo; folia angusta (<2 mm), perviridia, flaccida vel reflexa usque ad 40 cm longa; velum opacum, tegens plus minusve supremam decimam partem sporangii copiose brunneostriati; megasporae ±530 μm, obtectae latere distali ab ordinatione cristarum humilium et irregularium atque tuberculorum gracilium dense reticulata, latere proximali ab ordinatione iugorum et tuberculorum irregulari, et habentes zonam spinarum breviorum in latere iugi aequatorii distali; microsporae ±30 mm, vel parum obscure tuberculatae vel inornatae; chromosomata $2n = 66$.

FORM: Amphibious perennial herb arising from a 2-lobed corm, solitary or occasionally two joined at the corm; LEAVES: 20–25 (occasionally to 50), narrow (1.1–1.7 mm wide at midpoint), dark green when mature, 35–40 cm long, strongly reflexed or flaccid, flattened-oval in cross-section and occupied largely by four thin-walled air chambers (abaxial chambers slightly larger) and a central vascular bundle, becoming paler with narrow hyaline margins at the base; LIGULE: lanceolate-triangular, delicate, 3.0–4.5 mm long; VELUM: opaque, covering 9.45% (SD 1.56, N = 28) of the sporangium; SPORANGIA: ovate, ±6 × 4 mm, embedded into the basal adaxial side of the sporophylls; densely marked with light brown to dark brown streaks; MEGASPORES: white, 526.9 μm (SD 27.1, N = 79); with dense reticulate ornamentation pattern of irregular crests and thin tubercles on the distal side; irregularly radiating pattern of ridges and tubercles on the proximal hemisphere; short spines evident along the proximal ridges; dense band of short spines borders distal side of the equatorial ridge; spores are mature May–July (September ?); MICROSPORES: 29.8 μm (SD 2.28, N = 20), oval, plain or ornamented with small, low, sparsely distributed tubercles; CYTOLOGY: $2n = 66$. The epithet reflects the consistently short velum found over

the sporangia of this species, significantly shorter than that of all other polyploids in the southeastern United States.

PARATYPES: NORTH CAROLINA: Brunswick Co., Bolivia, Bolivia Branch of Middle Swamp, *A. E. Radford 5,187*, 2 Jun 1950 (NCU); *D. F. Brunton and K. L. McIntosh 12,599*, 3 Jul 1996 (OAC, DFB); *D. F. Brunton and K. L. McIntosh 13,227*, 29 Jun 1997 (OAC, DFB); Jones Co., Maysville, E bank of White Oak River, *D. F. Brunton and K. L. McIntosh 12,579*, 2 Jul 1996 (OAC, DFB); Onslow Co., Maysville, banks of White Oak River, *D. F. Brunton and K. L. McIntosh 12,213*, 23 Jun 1995 (OAC, DFB).

The following key to the species of *Isoetes* found along the southeastern coastal plain will assist in the discrimination of *I. microvela* from morphologically similar quillworts in that area. The megaspore figures represent the approximate average size from the specimens we examined and are provided as general guidelines. Discussions of more precise calculations of megaspore and microspore size variation can be found in recent literature concerning southeastern species, including Brunton and Britton (1996a, b, 1997), Brunton et al. (1994), Russell and Bray (1997) and Taylor et al. (1993). Size and morphological exceptions and extremes can be encountered with most of these taxa. Accordingly, *I. microvela* is keyed through each of the two main size and megaspore pattern options.

1. Megaspores >600 μm ..... (2)
   2. Megaspore ornamentation ±thin-walled; velum ±10% ..... *I. microvela*
   2. Megaspore ornamentation thick-walled; velum ≥30% ..... (3)
      3. Megaspore ornamentation coarse; ridges with thick walls in open pattern; velum ±60% ..... *I. georgiana*
      3. Megaspore ornamentation somewhat coarse; ridges with moderately thick walls in ±congested pattern; velum ±30% ..... *I. boomii*
1. Megaspores <550 μm ..... (4)
   4. Velum covering >50% of sporangium ..... (5)
      5. Leaves yellow-green, weakly erect to reflexed, 15–40 cm long; velum covering 50–70% of the unmarked sporangium; megaspore ornamentation

high-walled, with ragged-reticulate pattern .... *I. valida* (Engelm.) Clute

5. Leaves dark green, flaccid, 25–60 cm long; velum covering 80–100% of unmarked or sparsely brown-streaked sporangium; megaspore ornamentation of low, broad tubercles or ridges ... . *I. flaccida* Shuttlew.

4. Velum covering <50% of sporangium ..... (6)

6. Megaspores reticulate (evenly or irregularly) ... (7)

7. Megaspores ±450 μm; evenly reticulate ornamentation with ±thin walls; no equatorial band of spines ..... *I. engelmannii* (*s.str.*)

7. Megaspores >525 μm; unevenly reticulate ornamentation with ±thick walls; obscure to distinct equatorial band of low, thin spines ..... (8)

8. Velum 20–25%; megaspores with ±open, irregularly reticulate pattern; microspores with low tuberculate ornamentation ..... *I. appalachiana*

8. Velum ±10%; megaspores with ±congested, densely reticulate ornamentation pattern; microspores plain or sparsely ornamented with low tubercules ... . *I. microvela*

6. Megaspores tuberculate to irregularly and densely short-crested ..... (9)

9. Leaves 10–20 cm long; velum 25–30%; megaspore with moderately low, densely short-crested to tuberculate ornamentation; distinct equatorial band of short spines; outer shell (perispore) ±crumbly-surfaced; microspores with few, low, echinate tubercles .. .. *I. riparia* Engelm. ["*saccharata*"]

9. Leaves 20–45 cm long; velum <25%; megaspore with ±high, tuberculate to short-crested ornamentation; obscure to distinct equatorial band of short spines; perispore ± smooth-surfaced; microspores obscurely low tuberculate to conspicuously echinate ..... (10)

10. Megaspore ornamentation of tubercles and short crests; velum 15–20%; microspores densely echinate. . . *I. hyemalis*
10. Megaspore ornamentation short-crested to irregularly reticulate with few tubercles; velum 10%; microspores with obscurely tuberculate ornamentation . . . . . *I. microvela*

Origins. Most, if not all, North American polyploid *Isoetes* are believed to represent allopolyploids, as has been demonstrated for *I. riparia* (Taylor and Hoot 1997; Taylor et al. 1985) and *I. appalachiana* (W. C. Taylor, pers. comm). The hexaploid *I. microvela* could represent the allopolyploid product of the chromosome doubling of a sterile hybrid between a diploid and a tetraploid (*viz.*, $2x \times 4x = 3x$ [doubled] $= 6x$). Assuming that the resulting species would tend to reflect the morphological characteristics of both progenitor species, a number of *Isoetes* taxa occurring in the southeastern United States can be considered as candidate ancestors for *I. microvela*. These include diploids *I. engelmannii*, *I. flaccida*, and *I. valida*, and tetraploids *I. appalachiana* and *I. hyemalis*. Of the diploids, *I. valida* and *I. flaccida* seem unlikely to be involved since evidence of their large velum (50% or more) and densely echinate microspores (Brunton and Britton 1996b; Taylor et al. 1993) are not seen in *I. microvela*. If *I. engelmannii*, then, is the most likely diploid progenitor, we are left to determine whether *I. appalachiana* or *I. hyemalis* is the most probable tetraploid parent.

Were *Isoetes appalachiana* the tetraploid parent, one might expect an *I. microvela* megaspore pattern that is more evenly reticulate and which does not include tubercles. Although we did not employ ligule characteristics when considering possible progenitor species because of our limited understanding of this delicate structure, *I. hyemalis* appears to be the only other existing southeastern taxon with as large a ligule as *I. microvela* (J. Hickey, pers. comm.). The participation of *I. hyemalis* is also suggested by the occurrence of occasional *I. microvela* "sister" plants joined at their corms, a characteristic known otherwise amongst North American polyploids only with *I. hyemalis*. An origin involving *I. hyemalis* as the tetraploid progenitor, however, may not

be well supported by the obscure microspore ornamentation of *I. microvela*.

The combination of megaspore and leaf characteristics of *Isoetes microvela* described above is close to that of *I.* ×*bruntonii* Knepper & Musselman, the sterile triploid hybrid *I. engelmannii* × *I. hyemalis* (Musselman et al. 1996). On the basis of morphological evidence, therefore, *I. microvela* appears to represent an allopolyploid of *I.* ×*bruntonii* or possibly *I. appalachiana* × *I. engelmannii*.

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ADDENDUM. After the manuscript was in proof an additional population of *Isoetes microvela* was discovered along River Swamp, a tributary of Lockwood Folly River, 6.0 km south of Bolivia, Brunswick County, NC. Although severely wilted by prolonged exposure, scattered plants were observed growing with bryophytes (liverwort) and occasional graminoid seedlings through a dense mat of tree rootlets in fine sand on a creek bank 0.5-1.0 m above the present water level in a seasonally flooded swamp forest. A collection from this population (*D.F. Brunton and K.L. McIntosh 13,601*, 11 Jul 1998, OAC) was cytologically confirmed $2n = 66$.